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A vision of KIR variation at super resolution

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Abstract

The 9th killer cell immunoglobulin-like receptor (KIR) workshop was held in Winchester, United Kingdom late in the summer of 2015. The extraordinary diversity of KIR and its functional consequences were key themes throughout the meeting. Novel sequencing technologies and new bioinformatics techniques continue to increase our understanding of the genetic diversity and evolution of the KIR; whilst a deeper understanding of KIR functions, including their specificity for MHC and its peptide ligands, are generating more refined models of their role in disease. Limited to 100 delegates from around the world, this intimate workshop facilitated vigorous discussion, generating new ideas for research in this ever-expanding field.

A challenge that faces natural killer (NK) cells in fulfilling their myriad functions is adapting to the evolution of their MHC class I ligands. The selection pressure imposed on polymorphic MHC class I molecules by the need to adapt to pathogens tests the ability of the killer cell immunoglobulin-like receptors (KIR) to maintain a functional relationship with these ligands. They have met this challenge, in part, by rapidly diversifying. The diversity of KIR proteins was originally described in the 1990s, from analysis of NK cells expressing the DX9 epitope ¹. Subtle polymorphisms in *KIR* genes were noted in cDNA sequencing studies from several laboratories ²⁻⁴, but it wasn't until a genomic DNA analysis of 68 individuals was performed, that the extent of diversity of the *KIR* gene content began to be appreciated ⁵. In this study a 24 kB band on a southern blot divided the *KIR* haplotypes into *A* (encoding multiple inhibitory and one activating KIR) and *B* (encoding multiple inhibitory and activating KIR) types, with subsequent further refinement into centromeric *A* and *B*, and, telomeric *A* and *B* haplotypes ⁶. Disease association studies, especially for infectious disease and pregnancy syndromes, have

highlighted the role of diversity in *KIR* gene content in human disease. The recent and dramatic improvements in high-throughput and high-resolution sequencing technologies are giving new insight into the extent of diversity of the *KIR* gene family in humans. The 2015 *KIR polymorphism workshop* hosted *KIR* biologists from around the world to discuss the state-of-the-art for *KIR* biology, embracing *KIR* genetics, evolution, function and disease association.

The structure of *KIR* haplotypes, with their repetitive nature containing highly polymorphic genes with frequent copy number variation (CNV), has made sequence assembly and allele-level typing most challenging. As a consequence, knowledge of structural *KIR* diversity remains coarse, despite the growing need for better typing methods, particularly in the context of clinical transplantation. This state-of-affairs was highlighted by **Vierra-Green**, who showed how typing of common *KIR* haplotypes was largely successful. However this could also be challenging due to variation in the copy number of specific *KIR* genes, and more difficult in specific populations, such as Northern Native Americans. *KIR* typing of German bone marrow donor samples using amplicon sequencing shows promise, both in being cost effective and having the potential to yield allele information in the future (**Wagner** and **Lange**). However, high throughput methods may require refinement to accurately type more diverse *KIR* genes including *KIR3DL1/S1* (**Alicata** and **Moretta**). The improvement of *KIR* typing resolution is clearly an area where new technologies offer much potential. The ability of Pacific Biosystems sequencing to resolve the maternal and paternal *KIR* haplotypes, based on a fosmid library, was illustrated (**Hall** and **Ranade**) as well as more traditional methods like LinkSeq, a qPCR *KIR* specific detection mechanism (**Russnak** and **Antovich**).

Analysing what can be very complex sequence datasets is often a greater challenge than generating the data in the first place. Using pre-existing sequencing datasets offers an enormous resource. **Nemat-Gorgani** and **Parham** exemplified some of the analysis issues with a study of

divergent African populations. These populations contain a high frequency of novel *KIR* alleles and haplotypes that often precludes accurate genotyping. A combination of pyrosequencing, Sanger sequencing and Illumina technology enabled the discovery of several novel *KIR* variants predicted to have altered functionality, on a background of conserved telomeric *B* but highly diverse centromeric *B* haplotypes. Two methods were presented that use short read data to call *KIR* haplotypes by recognizing *KIR* specific reads, which are then used to call genotype and allele. A method named SeRRAMC was applied to a cancer cohort, correlating the absence of functional KIR2DS4 with greater risk of developing cancer (**Rossof and Lee**). Of particular note was the extremely high accuracy and rigour of using short reads in an *in silico* sequence-specific oligonucleotide probe approach, both for identifying known haplotypes and alleles but also in accurately constructing novel alleles sequences (**Norman and Parham**).

As diversification of *KIR* is not limited to humans, comparative studies to other catarrhine primates have considerable potential to inform evolutionary mechanisms and the scope of *KIR* diversity. The orangutan occupies an intermediate position in MHC and *KIR* evolution, with the main *KIR* ligand, MHC-C, emerging just prior to orangutan speciation. The structure of orangutan *KIR* haplotypes is similar to that in other great apes, but with greater diversity in the centromeric region, and relatively few *KIR* alleles shared by the Sumatran and Bornean orangutan species (**Guethlein and Parham**). Apart from catarrhine primates, the only other animals shown to have a diversified *KIR* gene complex are domestic cattle and their wild ancestor, the aurochs, in which a completely independent expansion of *KIR* genes occurred. Nevertheless, the similarities between cattle and primate *KIR* are striking, including significant polymorphism and a dominance of inhibitory receptors (**Gibson and Hammond**). However, cattle also have a highly diverse NK cell receptor repertoire based upon the C-type lectin-like receptors and it remains to be determined how this diversity translates into function (**Schwartz and Hammond**). In terms of the evolution of individual *KIR* genes, they come and go, as illustrated by the resurrection of the human-specific and highly polymorphic pseudogene *KIRDP1* (**Parham**).

KIR genes are expressed in a variegated fashion by NK cells, with both the frequency and levels of *KIR* expression exhibiting substantial donor variation. The control of variegated expression is an elaborate process, associated with a “probabilistic switch” in the *KIR* promoter. In a further refinement of his original model, **Anderson** demonstrated the influence of Pro1 elements on further controlling *KIR* expression in a tissue-specific manner. Control of cell-surface levels of *KIR* proteins can also be related to differences in the protein-coding region of the *KIR2DL5* gene (**Vilches**) and their context within the *KIR* locus (**Roberts** and **Riley**). Similarly, allelic variants of *KIR2DL1* are expressed on the cell surface at different levels, and they segregate on different *KIR* haplotypes (**Falco** and **Moretta**). The importance of the surface levels of *KIR* is that they affect NK cell function, with *KIR* analysis by stochastic optical reconstruction microscopy revealing that these levels can influence *KIR* clustering at the NK cell synapse (**Kennedy** and **Davis**). Furthermore NK cell inhibition is further impacted by the ability of zinc ions to mediate *KIR* polymerization (**Rajagopalan** and **Long**). *KIR* expression can also affect disease outcomes. Thus the chemotherapeutic agent 5-azacytidine used to treat myelodysplastic syndrome affects *KIR* expression and as a consequence augments the anti-leukaemic effect of NK cells (**Sohlberg** and **Malmberg**).

The gross specificity of the inhibitory *KIR* that recognize HLA-A, -B and -C epitopes alleles has been known for two decades, but the fine specificity of the inhibitory *KIR* for peptide:MHC complexes and the specificity of the activating *KIR* continues to attract the attention of *KIR* researchers. **Parham** reported that *KIR2DS5* can be an activating receptor specific for the C2 epitope of HLA-C, but this ligand specificity is exhibited only by some *KIR2DS5* allotypes. Thus, it is possible to correlate the protective effect of *KIR2DS5* against pre-eclampsia in Ugandans ⁷, with its ligand-binding specificity, demonstrating the importance of allele-level resolution *KIR* typing in studies of disease. The *KIR3DL1* gene is most diverse, and this diversity impacts upon its ligand binding (**Brooks**). Conversely the activating receptor *KIR3DS1*, which segregates as

an allele of the inhibitory receptor KIR3DL1, was found not to share an HLA-Bw4 specificity with KIR3DL1, but to bind to HLA-F, one of three non-classical MHC class I molecules (**Garcia-Beltran** and **Altfeld**). Structural studies have given deeper insights into allelic diversity, in terms of the subtle differences in the orientation of binding to HLA-C by KIR2DL2 and KIR2DL3 (**Pymm** and **Rossjohn**) and also the gross structural difference between KIR2DL4 and the other human KIR2D (**Moradi** and **Vivian**).

Challenging the concept of a simple motif-based ligand-binding model of the inhibitory KIR, **Sim**, **Boyton** and **Long**, showed data indicating that the HLA-C2 specific inhibitory receptor KIR2DL1 can bind group 1 HLA-C allotypes if an appropriate peptide is presented. Furthermore the 26 allotypes of KIR2DL1 and KIR2DS1 comprise four distinct phylogenetic clades (**Hilton** and **Parham**). With few exceptions, the members of each clade segregate with either *A* or *B* haplotypes and their ligand specificity for C1 or C2 allotypes correlates with the haplotype on which they are located. The enigmatic activating receptor KIR2DS2 can also bind HLA-C1 allotypes, but only in the context of specific peptides (**Khakoo**). Peptides derived from hepatitis C virus can also be presented by MHC class I to NK cells and thus induce KIR binding (**Lunemann** and **Altfeld**). This could be relevant *in vivo*, because the peptide-binding repertoire of MHC class I affects NK cell inhibition (**Mbiridindi** and **Khakoo**). For example, in HIV infection, **Holzemer** and **Altfeld** showed from sequence data analysis that *in vivo* an HLA-C binding peptide epitope mutates in a manner consistent with selection pressure by NK cells on HIV. Consistent with this model, lower levels of NK cell inhibition lead to increased killing of HIV-infected cells (**Korner** and **Altfeld**). The impact of the NK cell response on HIV is determined at the genetic level. Using a whole genome sequencing approach, **Carrington** described how specific KIR3DL1 alleles are associated with controlling HIV in the presence of one of its Bw4⁺ MHC class I ligands, HLA-B*57. Analysis of high-resolution genetic data from a

cohort of individuals chronically infected with hepatitis B, presented by **Hollenbach**, revealed a protective effect for *KIR3DL2* that was mediated only by some allelic variants.

Chronic viral infection, especially CMV, can lead to expansion of NK cells expressing particular KIR. **Uhrberg** showed how the *KIR A* and *KIR B* haplotypes can have differential expression of a shared *KIR* gene, again indicating the importance of the haplotypic context of each *KIR* gene in determining the expressed KIR repertoire. This has implications for bone marrow transplantation, as CMV-driven adaptive NK cells can have an augmented anti-leukaemic effect (**Miller**). New insights into the protective and susceptibility effects of KIR in a range of inflammatory and autoimmune disorders were described by **Rajalingam, Wellington and Elliott, Kollnberger and Bowness**, and, **Traherne and Trowsdale**. **Kusnierczyk, Chazara and Moffett** demonstrated that *KIR* genes are also important in the outcome of pregnancy). **Bjorkstrom, Sharkey, Ivarsson and Moffett** complemented these genetic models with phenotypic studies demonstrating the uniqueness of the uterine NK cell repertoire and the potential for differences in the education of uterine NK cells compared to peripheral blood NK cells. In particular, these studies have validated researchers' efforts to obtain difficult-to-collect human samples by giving novel insights into basic NK cell biology and opening up new ideas for NK cell-associated diseases.

Conclusion

For many years mechanistic understanding of the KIR variation underlying disease associations has been hampered by the low resolution gene-content typing of the extraordinarily complex *KIR* genes. This KIR workshop marked an exciting moment in time, by clearly demonstrating that recent gains in sequencing capacity and allele resolution now make population level *KIR* and *HLA* genotyping possible at the highest resolution. Combining these technological advances

with fine-resolution of KIR specificity and function now provides human immunogenetics with the opportunity for defining in complete detail the role of KIR, HLA class I and NK cell biology in the pathogenesis of infectious, inflammatory, neoplastic and pregnancy-associated diseases. Converting these ideas into treatment opportunities will be our next major challenge. The 10th KIR workshop will be organised by Francesco Colucci and will take place in Cambridge, UK in Spring 2017.

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